NORTHWEST FISHERIES CENTER
PROCESSED REPORT
OCTOBER 1975

REPORT OF PROGRESS ON A PILOT STUDY OF THE FEASIBILITY OF PRODUCING HIGH QUALITY SALMON FRY FROM ARTIFICIAL ENVIRONMENTS--1974 BROOD FRY PRODUCTION

by

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NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NOAA
U.S. DEPARTMENT OF COMMERCE

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METHODS AND TEST CONDITIONS

The hatchery facility, our methods of collecting and incubating eggs, natural spawning in Auke Creek, and our methods of sampling and processing fry are discussed below.

Building and Pipeline

A 24x44-foot heated building provided space for gravel incubators containing at least 1 million eggs, four stacks of tray incubators (Heath incubators)[2/], equipment for fry censusing and sampling, water bath tables in a room with seating for eight people to clip fins from fry, and a storage room. The building was built on the bank of Auke Creek near a fish counting weir where eggs could be collected from returning adult salmon.

Water from Auke Lake was supplied through a buried 4-inch polyvinyl chloride (PVC) pipe connected to a 14-inch wood stave pipeline. The intake was about 20 feet off the bottom and 20 feet beneath the surface of the lake during the incubation period, August 1974, to June 1975.

Water Quality

Water temperature and oxygen content were monitored during the 1974-75 test of gravel incubation. Water temperatures of Auke Creek surface flow and the incubator effluent were read once daily to the nearest 0.1^{o} C. with a mercury thermometer. Dissolved oxygen values of the water supply and the incubator effluents were measured to the nearest 0.01 mg/liter by the oxygen polarograph method at irregular intervals. The temperature and dissolved oxygen of the hatchery water supply were markedly different for incubation of the 1974 brood pink salmon than for previous broods. This year's unusually late appearance of an ice cover on Auke Lake because of unusually mild weather left Auke Lake (the water source for the hatchery) open to the circulating effects of winds until 5 January. The cooling and aerating of the lake partly corrected the deficiencies in the hatchery's water quality that have been blamed for the early emergence and slightly smaller size of the hatchery fry as compared to wild fry. Ice generally seals Auke Lake in October or November when the entire water column is a uniform 4^{o} C. and the oxygen content is about 9 mg/l (68% saturation). This winter, water temperature at freeze-up was 2.5^{o} C (Figure 1) and the oxygen level 11 mg/l (80% saturation) on 30 January dropped to 10.6 mg/l (78% saturation)

2/ Reference to trade names does not imply endorsement by the National Marine Fisheries Service.

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BACKGROUND AND OBJECTIVES

The National Marine Fisheries Service (NMFS) and the Alaska Department of Fish & Game (ADF&G) agreed in August 1971 to work together at the Auke Creek Experiment Station to test an upwelling gravel incubation system of producing high quality salmon fry which could be used to enhance or rehabilitate depleted stocks. The agreement is subject to annual approval by both agencies. Auke Creek was selected as the site for the tests because it is near the Auke Bay Fisheries Laboratory and because an existing pipeline provides an adequate water supply from nearby Auke Lake. Pink salmon, which spawn naturally in Auke Creek, were chosen because they have the shortest life cycle of all Pacific salmon. Pink salmon develop from egg to spawning adult in two years. Techniques for the production and release of pink salmon fry would also apply to chum salmon and sockeye salmon because extensive natural rearing areas that are presently underutilized by these species can be identified in Alaskan estuaries and lakes.

The pilot production and release of pink salmon fry at Auke Creek appear to be accomplishing the intended objectives of enhancing the adult run. The release of 187,600 hatchery fry and 157,000 wild fry from a parent escapement of 2,090 adults in 1971 resulted in a record run of 4,948 adults in 1973. The release of one-half million hatchery fry and 74,000 wild fry from a parent escapement of 1,768 adults in 1972 resulted in an even higher record of 6,260 adults in 1974. More than a million fry were released from eggs of the 1973 and 1974 broods, respectively, to complete the planned test of gravel incubators. The magnitude, timing, and other biological characteristics of returning adults in 1975 and 1976 will dictate whether additional tests with gravel will be needed.

Gravel is considered by developers of hatcheries in Alaska to be too expensive for wide-scale use because of its weight and bulk. Attention has focused on a plastic landscaping surfacing material, Astroturf [1/] which appears to have good potentials. Exploratory testing with turf began in 1973 and intensive testing began in 1974 at Auke Creek.

This report of progress concerning the 1974 brood pink salmon at Auke Creek describes the hatchery technology used and compares numbers, size, stage of development, and timing of emergence of unfed fry produced in various incubation environments including natural redds.

1/ Astroturf Landscape Surface, Type CH-4, made by Monsanto Company, 800 N. Lindberg Boulevard, St. Louis, MO 63166. Reference to trade name does not imply endorsement by the National Marine Fisheries Service, NOAA.

on 30 March. The water temperature remained at 2.5 to 2.6^{O} C until 24 April, then gradually rose to about 49^{O} C during the next 30 days.

This year we experienced repeated reductions in flow to several of our small incubators caused by sticklebacks that entered the main water supply from Auke Lake. Sticklebacks had been noticed in the hatchery water supply in previous years but they did not block flows through the large gravel incubators.

Collection of Adults and Pretreatment of Eggs

All eggs for the 1974-75 test were obtained from Auke Creek pink salmon. Adult pink salmon began entering Auke Creek July 24, 1974, and continued until October 2. The adults were enumerated and sorted several times daily as they entered the trap at the Auke Creek weir. Approximately one-fourth of the entering females plus an equal number of males were placed in holding pens each day. The remaining adults were released above the weir so that they could spawn naturally. We avoided assortative selection of spawners by alternately placing fish in pens or releasing them in the creek without regard to size or other physical characteristics. The adults generally had to be held in pens about 5 days to allow their eggs to ripen for artificial spawning. Eggs were collected from 639 females between August 9, and September 18. A sample of 25 females had an average egg content of 2,198 eggs per female (standard error of the mean, 51 eggs). The eggs were taken by incision after the females were killed by a blow on the head and bled by cutting the caudal artery; care was taken to avoid contamination of the eggs with blood, slime, or water. The females were spawned individually into a clean and dry 1-gallon plastic pail and the contents immediately were divided equally into 5 to 7 aliquots by pouring them into 5 to 7 clean, dry pails. As soon as each of the 5 to 7 pails contained the equivalent egg content of one female, the eggs were fertilized by expressing milt into them from freshly killed males. A separate male was used for each pail and only one male was used for each pail, except where there was doubt about the quantity or quality of milt. A second male was used if the first had yielded only a few drops of milt, watery milt, or bloody milt. The eggs and sperm were then gently mixed and washed with fresh water. The wash water was a 50-50 mixture of creek water and hatchery water. We took the precaution of mixing water to reduce temperature shock to eggs and sperm because the creek water was 5 to 9^{O} C warmer than the hatchery water during the spawning season. The fertilized eggs were then poured into the trays of Heath incubators. Each tray received the eggs from about 14 females. The Heath incubators in which the eggs were eyed were covered to exclude light. Malachite green treatments (15 ppm for 1 hour at 10-day intervals) were used to control fungus growth. Dead eggs were removed and the live eyed eggs were seeded into the gravel incubators in October and November.

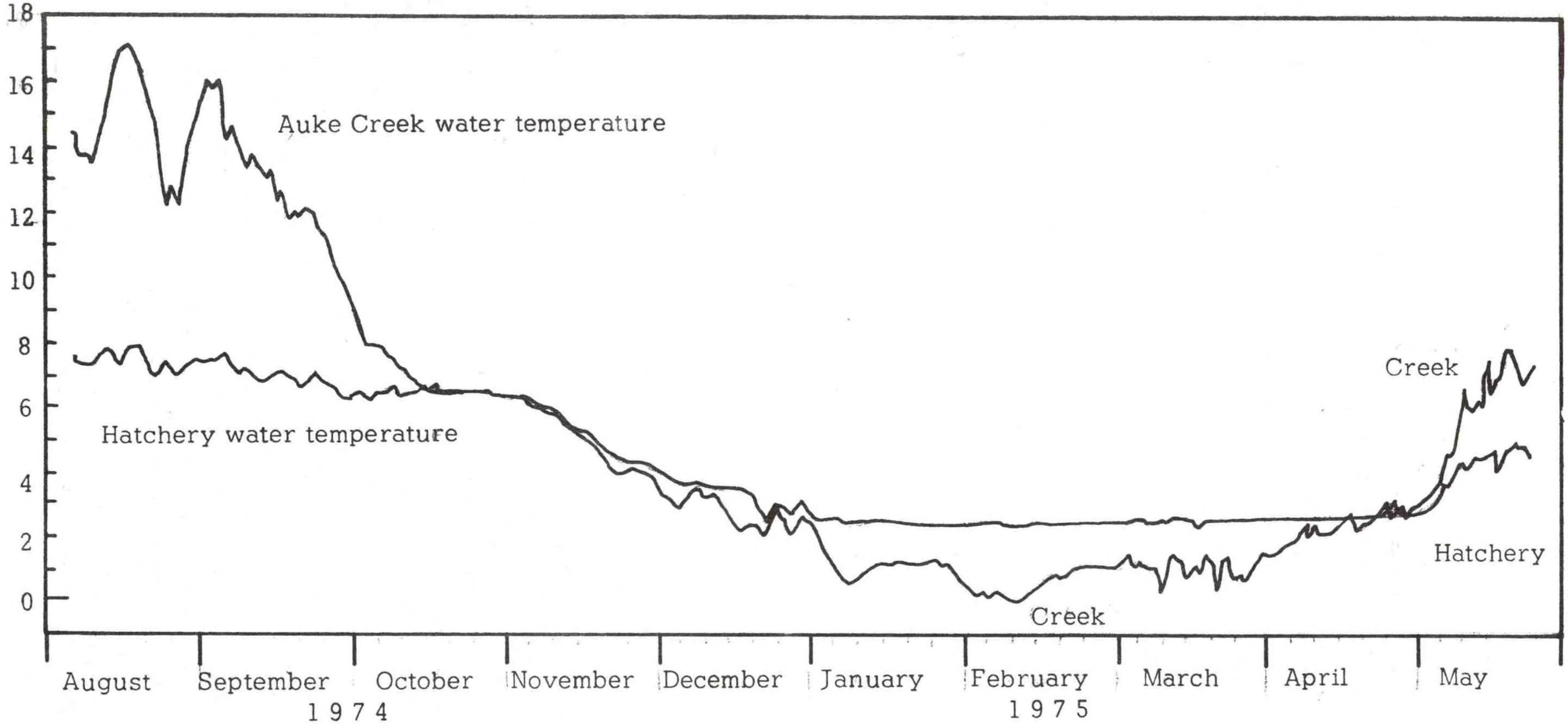

Figure 1.--Water temperature in the Auke Creek Hatchery and surface water temperature of Auke Creek adjacent to the hatchery during the incubation season, 1974-1975.

Length (mideye to tail-fork length) and total wet weight measurements were taken from 720 adults in 1974. The egg contents of 25 females were hand-counted in 1974 to determine fecundity. Sex ratios were determined by counting all the fish of each sex when sorted at the weir.

Incubators

The incubators tested at the Auke Creek Hatchery this year can be classified into two basically different types which we call upwelling-flow incubators and horizontal-flow incubators.

Upwelling-flow incubators have a forced, or direct flow of water through a matrix or substrate that supports the eggs or alevins. The matrix which may be gravel or some other porous material such as Astroturf, may range in depth from about 1-inch to 4 feet. The modified Bams box, which we used this year, is an upwelling-flow incubator as are the layered turf, coiled turf, and Heath trays with gravel. Horizontal-flow incubators usually have only about 1 inch of substrate depth, such as a single layer of gravel or a single layer of plastic turf, and the water flows horizontally over the surface with no provision for a forced flow through the substrate. The roughness of the substrate is expected to induce some movement of water into the pore spaces of the substrate. Our modified Heath trays, described in this report, and the Auke Bay Incubators are horizontal-flow incubators.

Upwelling Gravel Incubator

The 5 gravel incubators seeded with pink salmon eggs in 1974 (Table 1) were 4x4x4 feet deep. The boxes were coated with black pigment to exclude light from entering the sides and bottoms and the tops were left uncovered. The surface area of gravel was 16 feet^2, but each box occupied about 20 feet^2 of floor space. Water was introduced through a false bottom of perforated sheet stock. The false bottom was a sheet of perforated polypropylene plastic 1/8 inch thick with 3/32-inch holes drilled on 15/32-inch staggered centers. The perforated plate was supported on 3/4-inch diameter by 1-3/16-inch long polypropylene rods on 6-inch centers. The 3/4-inch to 1-1/4-inch river gravel was washed to remove all particles under 1/2 inch and over 2-1/4-inch in diameter. The gravel was shoveled by hand from a wheelbarrow into the boxes and the eggs were seeded in 8 layers, with 4 inches of gravel between layers of eggs and 4-to-5 inches covering the top layer of eggs. About 3 inches of open water was left at the top so that fry could swim to the outlet on the side of the box. Seeding density was therefore about 1.6 eggs/in.^{3} of gravel. A strong flow of water was maintained during the loading

Table 1.--Type of test incubators, number of each type and operating characteristics of incubators that were seeded with 1974 brood pink salmon eggs at Auke Creek

Incubator type	Units seeded (number)	Eggs/in.^{3} of substrate (number)	Rate of flow/m^3 of substrate (gpm)	Apparent velocity (cm^3/hr/cm^2)	Wt. of fry/gpm at 100% survival (lbs.)*
		Upwelling Flow Incubators			
Upwelling gravel	5	1.6	13.0	300	4.3
Layered Astroturf	1	6.5	52.5	215	4.3
Coiled turf	1	4.8	38.5	290	4.3
Heath trays w/gravel	13	40 to 80	2836	1200	7.5
		Horizontal-Flow Incubators			
Modified Heath trays with gravel	10	40	2836	--	4.9
Auke Bay Incubator density-flow test	10	23 to 76	347 to 926	--	0.9 to 2.3
Auke Bay Incubator vertical stack	9	20	578	--	10.8

* Average weight of fry - 259 mg.

operation so that the upwelling water carried off some silt. As soon as the seeding was completed, the flow to each box was reduced to 20 gpm. This flow resulted in an apparent velocity of 300 $cm^3/hr/cm^2$ in the incubator. Volitional emergence of the fry was permitted and monitored daily.

Layered Astroturf Incubator

An exploratory test was conducted with a small box containing 8 layers of Astroturf instead of gravel substrate. This was a repeat of a test last year with Astroturf and 1973 brood pink salmon eggs. The transparent incubator was 10x20x30 cm deep. It was seeded with 6.5 eggs/$in.^3$ of turf and received 716 ml/min. flow of water. Apparent velocity was 215 $cm^3/hr/cm^2$. The incubator was loosely covered with a sheet of black plastic to reduce exposure to light. Volitional emergence of the fry was monitored daily.

Coiled Turf Incubator

A test with Astroturf involved a single incubator with upwelling flow through a coil of turf as suggested by Mr. Robert Dewey. A 13-inch wide strip of turf was loosely coiled and the coil placed on end over a perforated plate near the bottom of a 5-gallon plastic garbage can. Seeding density was 4.8 eggs/$in.^3$ of turf (Table 1) and the eyed eggs were placed on the top surface of the coil about two weeks before they hatched. The coil of turf was 15.75 inches in diameter, water flow was 1.6 gpm, and apparent velocity was 290 $cm^3/hr/cm^2$. Volitional emergence of the fry was monitored daily.

Heath Trays with Gravel

Thirteen Heath trays were lined with a single layer of 1/2 to 1-1/4 inch gravel and seeded with eyed eggs. Each tray held about 150 $in.^3$ of gravel. Ten of the trays were seeded at the rate of 40 eggs/$in.^3$; 2 trays with 66 eggs/$in.^3$; and 1 tray with 80 eggs/$in.^3$ (Table 1). Water flow to this stack of trays was maintained at 7 gpm. Volitional emergence was not possible from Heath trays operated in this manner because the fry were locked inside the baskets. The fry were released on the date that we visually determined that the majority were "buttoned up."

Modified Heath Trays with Gravel

Ten Heath trays were modified and seeded with eyed eggs to determine whether this incubator could be readily adapted to allow volitional emergence of fry. The modification involved removal of the egg baskets with their covers and cementing a baffle across the upstream end of the tray. A single layer of 1/2 inch to 1-1/4 inch gravel, about 150 in.^{3} of gravel, was placed in the tray. The baffle induced water to flow horizontally across the gravel as in shallow matrix incubators. Water flow was maintained at 7 gpm and the trays were seeded at the rate of 40 eggs/in.^{3} of gravel (Table 1). Alevins that flushed out prematurely were caught beneath the basket of a standard Heath tray at the bottom of the stack. These "flush-out" alevins were returned to the top of the stack each morning. The fry were released on the date we visually determined that the majority were "buttoned up."

Auke Bay Incubator--Density Flow Test

Ten of the Model 2, Auke Bay Incubators designed by Salter (1975) were operated with independent water supplies, seven factor combinations of flow and density. This incubator had a horizontal flow of water about 3 inches deep over a single layer of Astroturf. Eyed eggs were seeded into hatching screens which were suspended into the water above the turf. Newly hatched alevins dropped through the screens to continue their development on the turf. Each incubator had 527 in.^{2} of turf (dimensions, 14.75 in. x 35.75 in.). Seeding densities and water flows were varied as shown in Tables 1 and 2. The parallel rod separators were used as a screen to prevent premature flushing out of alevins. The separators were not entirely satisfactory for this purpose and alevins escaped from some incubators. The separators were placed in their normal position to allow volitional emergence only after we had determined that most of the fry were "buttoned-up."

Table 2.--Water flows and seeding densities in a test of ten model 2 Auke Bay Incubators with pink salmon eggs, 1974 brood

Eggs seeded (number)	Flow rate (gpm)	Incubators (number)
12,121	3.0	1
30,039	3.0	1
3,162	5.5	1
21,080	5.5	4
39,998	5.5	1
12,121	8.0	1
30,039	8.0	1

Auke Bay Incubator--Vertical Stack Test

A stack of nine Model 2, Auke Bay Incubators, was seeded with eyed pink salmon eggs (Table 1) and supplied with 5 gpm flow to test the areation characteristics of this incubator and to compare fry from this incubator with fry from other environments. A tenth incubator at the top of the stack served as a cover and was not seeded with eggs. The incubators were seeded at the rate of 20 eggs/in.^{3} of turf. Premature flushing out and emergence of fry were controlled with the parallel rod separators the same as in the density-flow experiment.

Natural Spawning

A total of 6,260 adult pink salmon entered the fish counting weir from July 24 to October 2, 1974, About 79%, 2,348 females and 2,584 males, were released to spawn above the weir. The remainder were retained as a source of spawn and samples for fecundity counts. Most pink salmon spawned in the 297 m. section of stream between the weir and Auke Lake. A few adults spawned in Lake Creek above Auke Lake. The Auke Creek streambed above the weir had a total area of approximately 1,430 m^2 of which only 60% or 860 m^2 was suitable for spawning. The remaining 40% of the streambed was bedrock and boulders. Pink salmon also spawned in the intertidal zone below the weir but we did not estimate their number. A hydraulic pump census (McNeil, 1964) in March revealed that about 24% of the natural pink salmon alevin population of Auke Creek was in the intertidal zone. The section of intertidal streambed utilized by pink salmon was 75 m. long and had a total area of 556 m^2, of which 256 m^2 was suitable for spawning. In previous years we had reported 330 m^2 was suitable for spawning but the 74 m^2 pool area below the weir has consistently failed to produce fry. For this reason we no longer include this pool in the intertidal census.

Collecting and Processing Fry

Migrant fry from the test incubators and from Auke Creek above the weir were monitored daily to count or index their numbers, obtain samples for size measurements and fin clip a number from each type of incubation environment.

A 3'x3' fyke net with floating livebox was operated from February 25 through June 14 to index the emigration of creek fry and to fin clip a portion of the creek fry. Heavy precipitation and runoff caused us to remove the fyke net for four days, May 11-14. A total of 39,320 fry were caught.

The daily counting of fry as they emerged from gravel incubators, A, B, C, and D was expedited by passing the incubator effluents over a cone-shaped sampling device.3/ The cone of the sampler rotated at the rate of about 8.5

3/ A blueprint for the cone-shaped fish sampler was supplied by the Washington Department of Fisheries.

rpm. The sampler retained 4.25% of the fry for hand counts and fin clipping and allowed the remainder to pass. Fry from these gravel incubators that passed the cone sampler were routed directly into the hatchery drain and thence into Auke Creek. All of the fry from gravel incubator G; from the vertical stack of turf incubators and from the coiled turf incubator were retained each day for counting and fin clipping.

Fry from all remaining incubators were either hand-counted daily or when their numbers exceeded 1,000 or they were inventoried by wet weight. Duplicate samples of about 500 fry per sample were used to convert total wet weight of each such population of fry to an estimate of the number of fry. All fry that were retained for counting or fin clipping were released at 11:30pm of the same day. Twice weekly, samples of 50 fry from each incubator permitting volitional emergence and from the fyke net were preserved in 5% formalin. Duplicate samples of 50 fry per sample of the Heath incubator fry were preserved on the date of release. The preserved fry were allowed to stand for 6 weeks before lengths were measured to the nearest millimeter and wet weights to the nearest milligram. An index to stage of development (Bams 1970) of the fry was computed from the formula:

$$K_D = 10\sqrt[3]{\frac{\text{length in millimeters}}{\text{weight in milligrams.}}}$$

This index is used only on unfed fry to indicate the relative yolk content. It is not a condition factor. In an earlier test (Bailey and Taylor, 1974) the average K_D index for incubator fry was 0.016 unit higher than the index for creek fry. We interpreted this to mean incubator fry emerged about 3 days earlier in stage of development or yolk assimilation than creek fry.

Weighted means and variances of pooled data were computed on the basis of the fraction of the migrant fry represented by each sample. Statistical comparisons were made of lengths, wet weights, and developmental index as follows:

$$\bar{Y}_w = \sum W_i \bar{Y}_i,$$

where

$\bar{Y}_w$ = weighted mean,

$\bar{Y}_i$ = observed mean measurement in ith period,

W_i = proportion of run leaving in ith period from index sampling

$$\widehat{V(\bar{Y}_w)} = \sum_{i=1}^{n} W_i^2 \widehat{V(\bar{Y}_i)},$$

where

$V(\bar{Y}_w)$ = variance estimate of weighted mean,

$V(\bar{Y}_i)$ = sample variance of estimated mean in ith period,

n = number of periods sampled.

A crew of part-time technicians marked anesthetized fry by clipping the adipose and left ventral fin from hatchery fry , or adipose and right ventral fin from fyke-netted creek fry. The anesthetic solution, 1:7, 500 MS-222 (tricaine methanesulfonate) buffered with sodium bicarbonate to pH 6.1-6.4, was recirculated and kept cool in a water table. Surgical iris scissors were used to excise fins under a 3X magnifying lens. Markers with 3 days experience demonstrated the capability to fin clip fry at an average rate of at least 200 fry per hour. The average speed for the entire season was about 100 fry per hour when figured on the basis of total hours paid, because the fin-clippers were assigned to fry counting duty about half of the time. Samples of fry from each marker were examined several times each day to ensure that the correct fins were excised as close to the body as possible. All marked fry were released at 11:30pm the same day they were marked. Dead fry remaining in the release tank were counted each morning. This immediate mortality from marking was less than 0.1% of the fish marked. The marking of hatchery fry began in March and was completed in May. Creek fry were marked between April and May.

RESULTS

The number of fry produced in our incubators was about four times the number of fry produced from natural spawning in Auke Creek above the weir. We compared length, weight, stage of development and timing of emergence of migrant fry from the incubators and from the creek. Except for fry needed to evaluate size and stage of development, fry from the hatchery were released to enhance pink salmon production in Auke Creek.

Survival from Egg to Fry

We spawned 639 females and obtained 1,301,800 green eggs, or 93% of their estimated contents of 1,404,500 eggs. A total of 1,233,894 eggs (Table 3) or 95% of the eggs we collected attained the eyed stage of development in the Heath incubators. The eyed eggs were then seeded into the test incubators and 89% of those incubated in the hatchery attained the emergent fry stage. We preserved 8,400 fry for size measurements and released 1,078,798 fry into Auke Creek. Marked fry included in the total released were:

Mark	Source	Number of Fry
AD-LV	Gravel Incubator G	27,519
AD-RV	Creek	18,078
D-LV	Gravel Incubator G	27,452
D-RV	Turf Incubator	6,561

Table 3.--Disposition and fertilization dates for eyed pink salmon eggs and fry production at the Auke Creek Hatchery in fall 1974

Incubator Type	Eggs Fertilized (Date)	Units (Number)	Eyed Eggs Seeded (Number)	Fry Produced (Number)
Upwelling gravel: A	Aug. 9-26	1	150,000	139,747
Upwelling gravel: B	Aug. 26-30	1	150,000	137,639
Upwelling gravel: C	Aug. 30-Sept.9	1	150,000	137,335
Upwelling gravel: D	Sept. 9-11	1	149,000	129,534
Upwelling gravel: G	Sept. 11-18	1	150,000	143,764
Upwelling Astroturf	Sept. 11-18	1	1,424	1,338
Coiled turf	Sept. 11-18	1	12,100	5,536
Auke Bay Incubator Density Flow-test	Sept. 11-18	10	210,800	171,533 1/
Auke Bay Incubator Vertical Stack test	Sept. 11-18	9	94,320	78,802
Heath trays with gravel	Sept. 11-18	13	92,000	89,590
Modified Heath trays with gravel	Sept. 11-18	10	60,000	52,380
Oxygen Uptake and Oil Bioassays (Rice)	Sept. 11-18	--	12,000	0
Physiology Studies (Helle)	Sept. 11-18	--	250	0
		Total	1,233,894	1,089,198

1/ Apparent loss of fry due to premature flush-outs which were not returned to the incubators.

About 61% of the eyed eggs were seeded into the 4'x4'x4' feet deep gravel incubators; 26% in Astroturf incubators; 12% in Heath incubators and 1% were allocated to studies outside the Auke Creek Hatchery. Survival from eyed egg to emergent fry was 92% in the 4x4x4' deep gravel incubators; 81% in Astroturf; and 93% in Heath incubators. The lower survival in Astroturf was not due to death of alevins in the turf but was really the result of premature flushout of uncounted alevins. Overall survival from eggs in females to emergent fry for all incubators combined was 77%. Comparable survivals for previous brood years at the Auke Creek Hatchery were: 1971, 69%; 1972, 75%; and in 1973, 79%.

The census of 96 random points with a hydraulic pump in March 1975 indicated that 268,320 fry resulted from natural spawning in Auke Creek above the weir. The egg contents of the 2,348 females that spawned above the weir was 5,161,000 eggs. Survival in the stream was therefore only 5.2% compared to 77% in the hatchery. Comparable survivals for previous brood years were: 1971, 12.1%; 1972, 7.9%; and 1973, 9.3%. The low survival in freshwater for the 1974 brood was probably the result of excessively high spawner density which reduces the efficiency of spawning.

Fry Size and Stage of Development at Emergence

Samples of fry collected in spring 1975 were used to compare our gravel incubator (Bams' Box) fry with creek fry, to explore effects of new incubator designs on fry, and to establish guidelines for proper seeding densities and flow rates for the Auke Bay Incubator.

Gravel Incubators

Fry from three of the five gravel incubators (Incubators C, D, and G) were equal to or larger than creek fry in length and weight (Table 4, and Figures 2 and 3), but fry from Incubators A and B were slightly smaller than creek fry. The average developmental index for creek fry was 1.98 compared to averages of 1.99 to 2.01 for these gravel incubators (Table 4 and Figure 4). This difference in average developmental index is interpreted to mean that the incubator fry migrated to sea about 4 days earlier in stage of development than creek fry.

Table 4.--Lengths, weights, and stages of development of unfed pink salmon fry at Auke Creek and Auke Creek Hatchery in spring 1975

Incubation Environment	Samples (number)	Length mean (mm)	Length variance (mm)	Weight mean (mg)	Weight variance (mg)	Developmental Index mean (K_D units)	Developmental Index variance (K_D units)
Creek	11	32.2	.002	257.9	1.44	1.979	0.30×10^5
Upwelling gravel							
Incubator A	7	31.2	.003	246.8	1.81	2.001	0.75×10^5
B	6	31.8	.004	254.3	2.39	1.987	0.78×10^5
C	4	32.0	.009	265.5	5.95	2.005	1.04×10^5
D	3	32.1	.016	263.3	8.45	1.996	2.11×10^5
G	5	32.0	.006	266.2	2.16	1.998	0.92×10^5
Layered turf incubator	4	31.9	.006	245.4	3.53	1.958	1.03×10^5
Coiled turf incubator	6	31.3	.005	238.9	2.45	1.984	0.82×10^5
Heath trays with gravel	6	32.1	.004	266.2	2.09	2.002	0.73×10^5
Modified Heath trays with gravel	2	31.5	.008	261.8	6.80	2.030	19.51×10^5
Auke Bay incubator vertical stack	6	32.2	.004	273.7	2.01	2.016	0.75×10^5

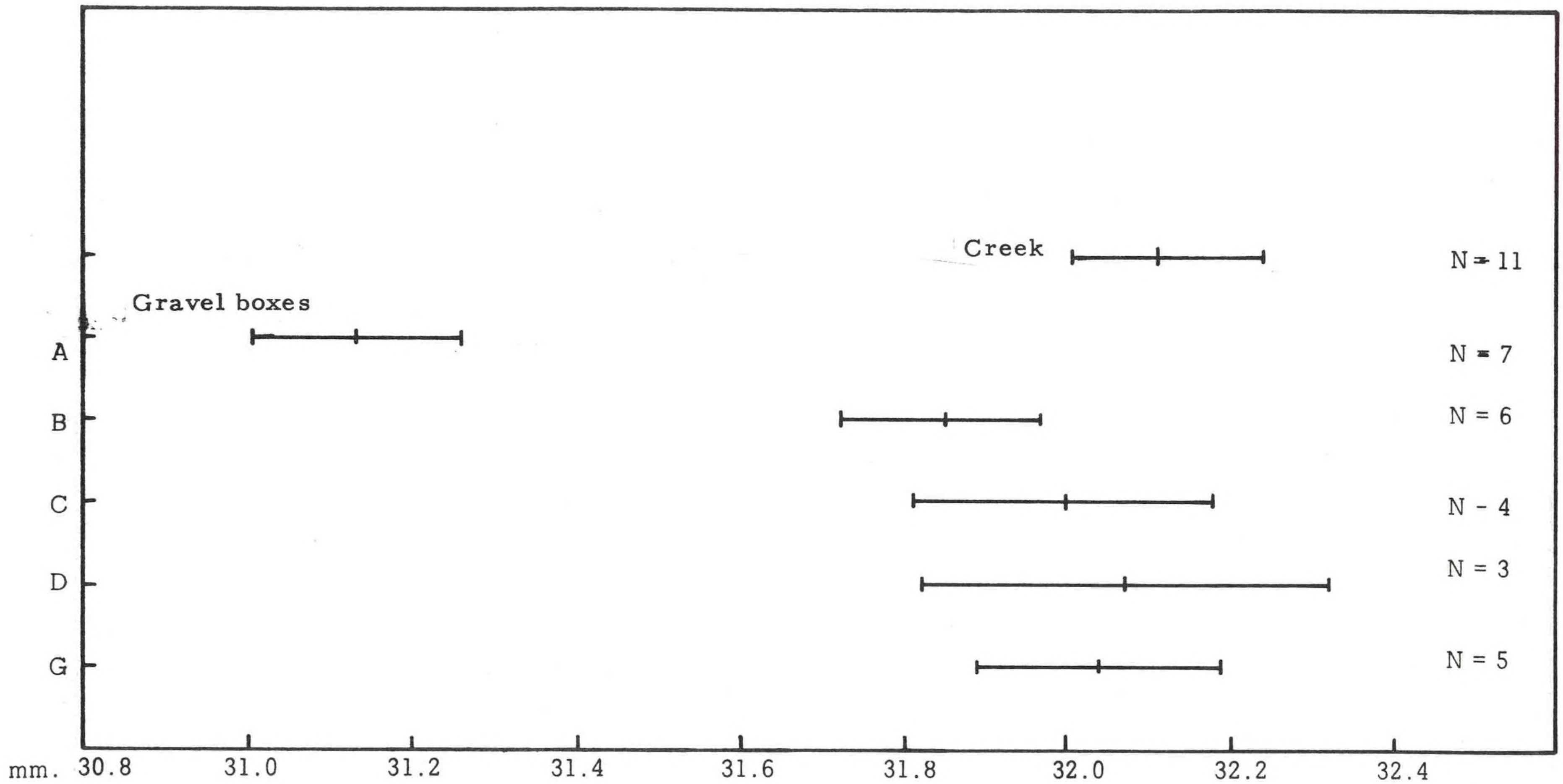

Figure 2.--Lengths of unfed pink salmon fry from Auke Creek and from 4'x4'x4' gravel incubators; the length of each line represents ± two times the standard deviation of pooled means for N samples of 50 fry per sample; 1974 brood.

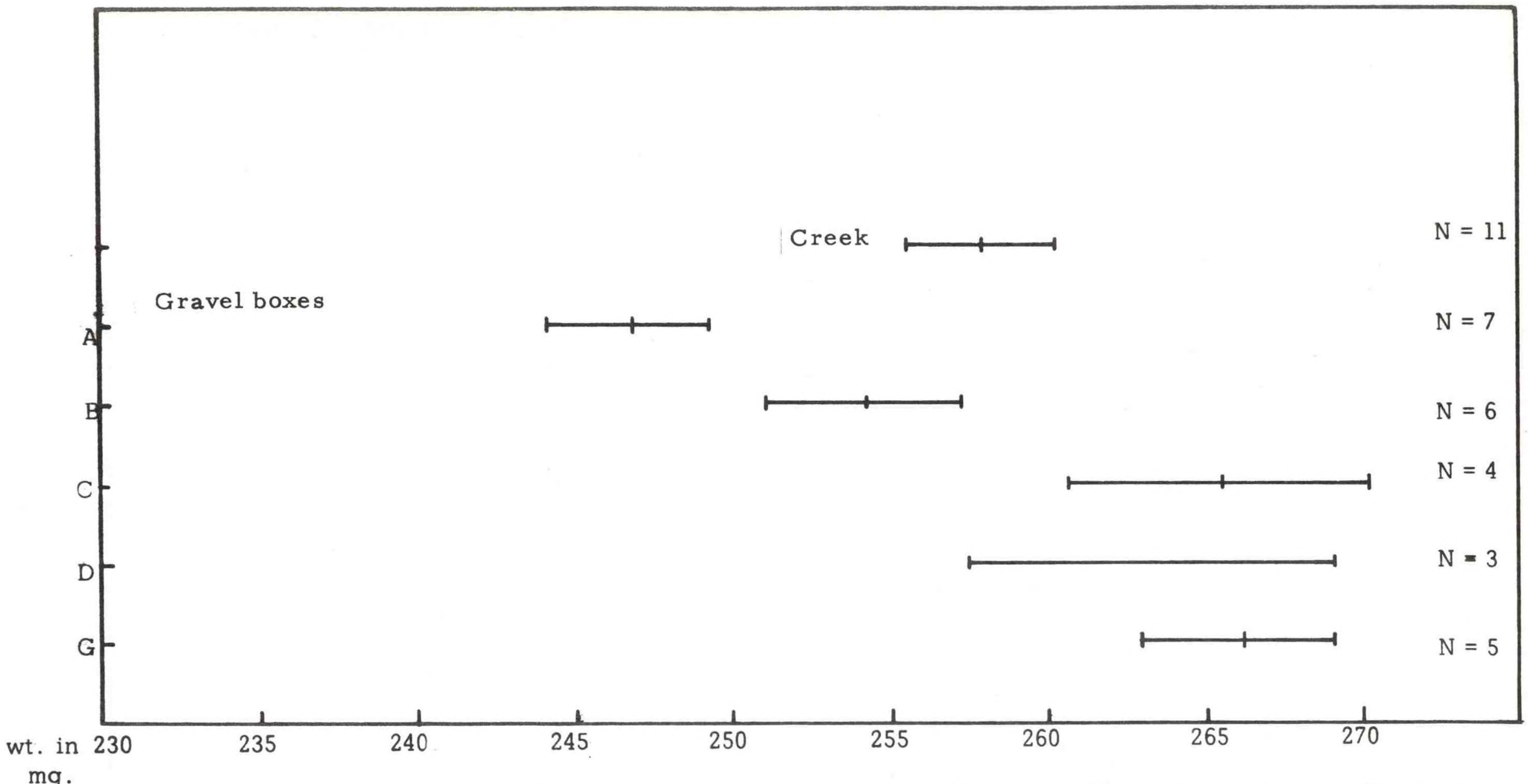

Figure 3.--Wet weights of unfed pink salmon fry from Auke Creek and from 4'x4'x4' gravel incubators. The length of each line represents ± two times the standard deviation of pooled means for N samples of 50 fry per sample; 1974 brood.

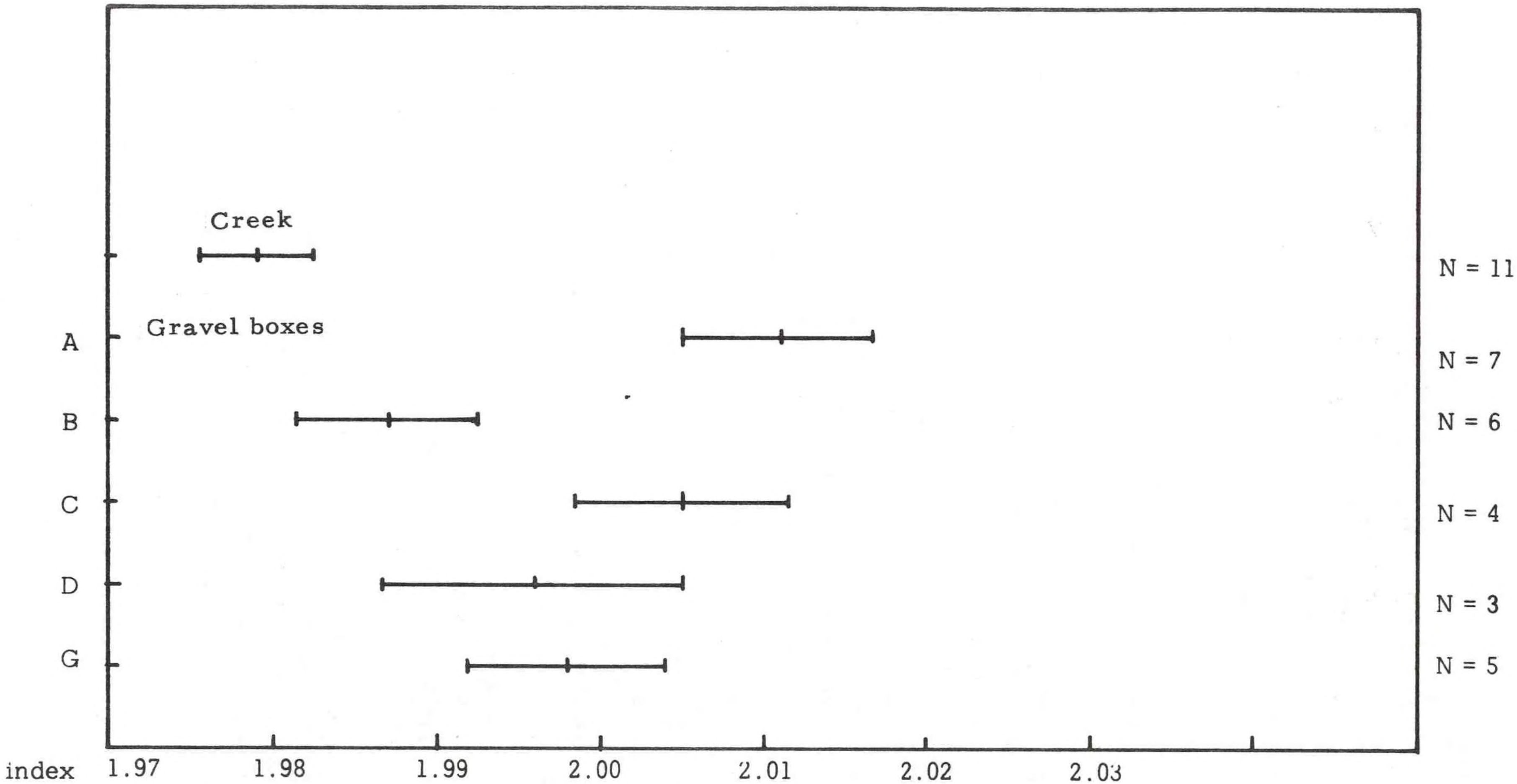

Figure 4.--Indices of development, K_D. of unfed pink salmon fry from Auke Creek and from 4'x4'x4' gravel incubators. The length of each line represents ± two times the standard deviation of pooled means for N samples of 50 fry per sample. 1974 brood.

New Incubator Designs

All fry from gravel Incubator G, all fry from the Turf Incubators, and all fry from the Heath trays originated from the same egg take. Differences in size and stage of development of emergent fry from these incubators can therefore be attributed to differences in the incubation environments. Incubator G, an upwelling-flow gravel incubator, represents a proven hatchery method for enhancing pink salmon runs through releases of unfed fry. Published reports favorably compare this method with natural spawning on the basis of both fry produced and adult returns. Fry from Incubator G therefore constitute a standard or control for evaluation of fry from the other incubators. The Turf Incubators and Heath trays used at Auke Creek represent exploratory attempts to eliminate or greatly reduce the need for gravel.

Fry raised in modified Heath trays were significantly shorter but not different in weight when compared to fry from Incubator G (Table 4 and Figures 5 and 6). The modified Heath tray fry were released at an earlier stage of development than the Incubator G fry (Table 4 and Figure 7). The difference, 0.032 K_D units, indicates these fry could have been held in the Heath trays at least another 6 days and their length would then have increased to equal that of Incubator G fry. Experience has shown that the K_D index decreases at a rate of 0.005 K_D units per day and the length increases at a rate of 0.08 mm/day during the last month of incubation.

Fry were raised in unmodified Heath trays with gravel at two densities, 6,000 eggs per tray and 10,000 eggs per tray. At the time of release, the fry at the lower density were equal in length, equal in weight, and equal in stage of development to Incubator G fry. The fry at the higher density were longer in length, equal in weight, and later in stage of development than Incubator G fry. Apparently the alevins responded to the higher number of eggs per tray by accelerating their development.

Fry produced in the vertical stack of nine Auke Bay Incubators were equal in length, heavier in weight, and earlier in stage of development by 0.018 K_D units than fry from Incubator G. Had they been held another 3 days in the stack, their length could have exceeded that of fry from Incubator G.

The coiled turf and layered turf incubators produced fry that were equal in length, lighter in weight, and later in stage of development than fry from Incubator G. Only a single incubator of each type was used and in each case there were circumstances that cast doubt on the validity of these results. Sticklebacks plugged the valve in the water supply to the coiled turf incubator

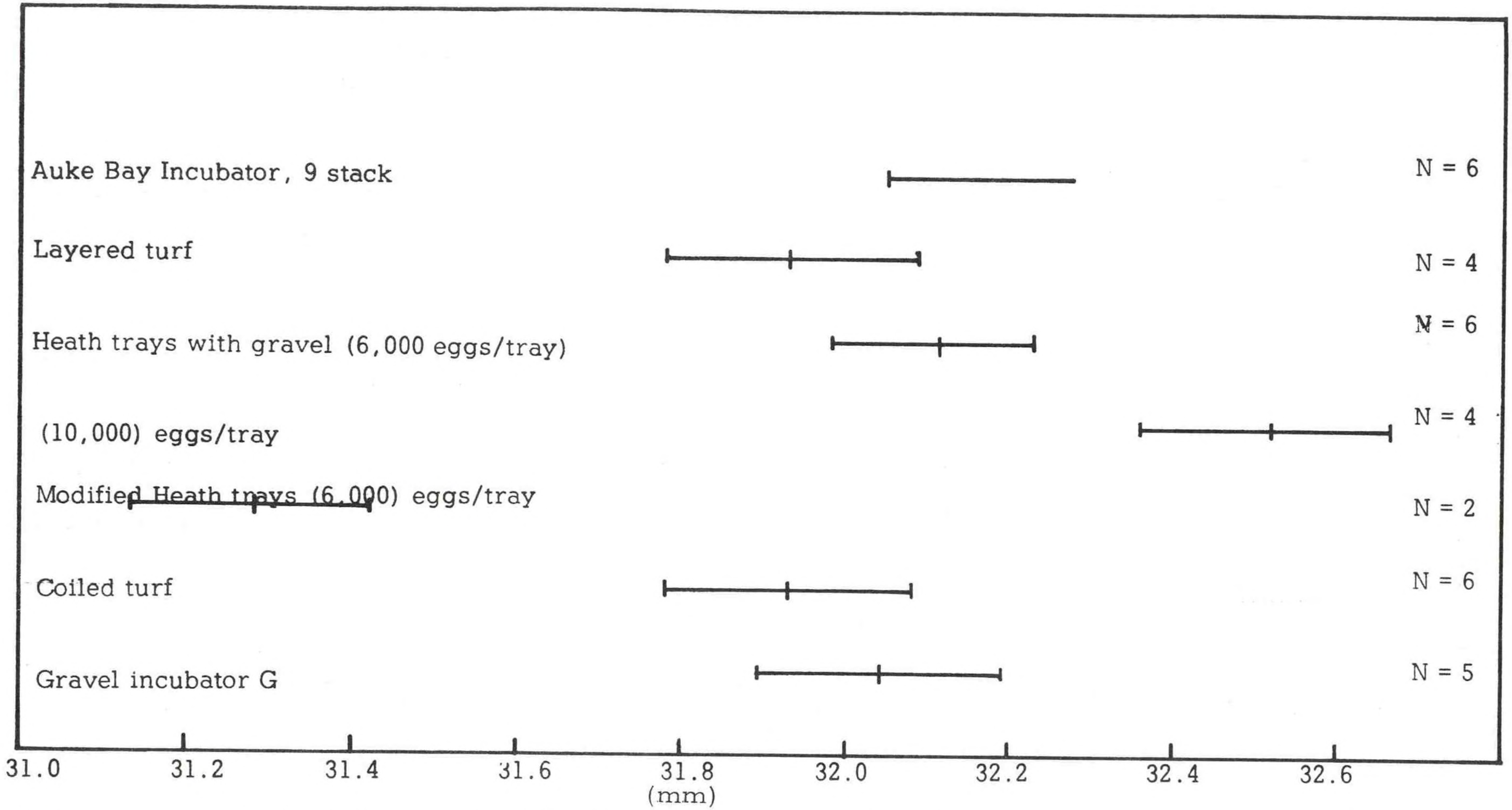

Figure 5.--Length of unfed pink salmon fry after incubating eggs from the same parents in incubators of varying designs; the length of each line represents ± two times the standard deviation of pooled means for N samples of 50 fry per sample.

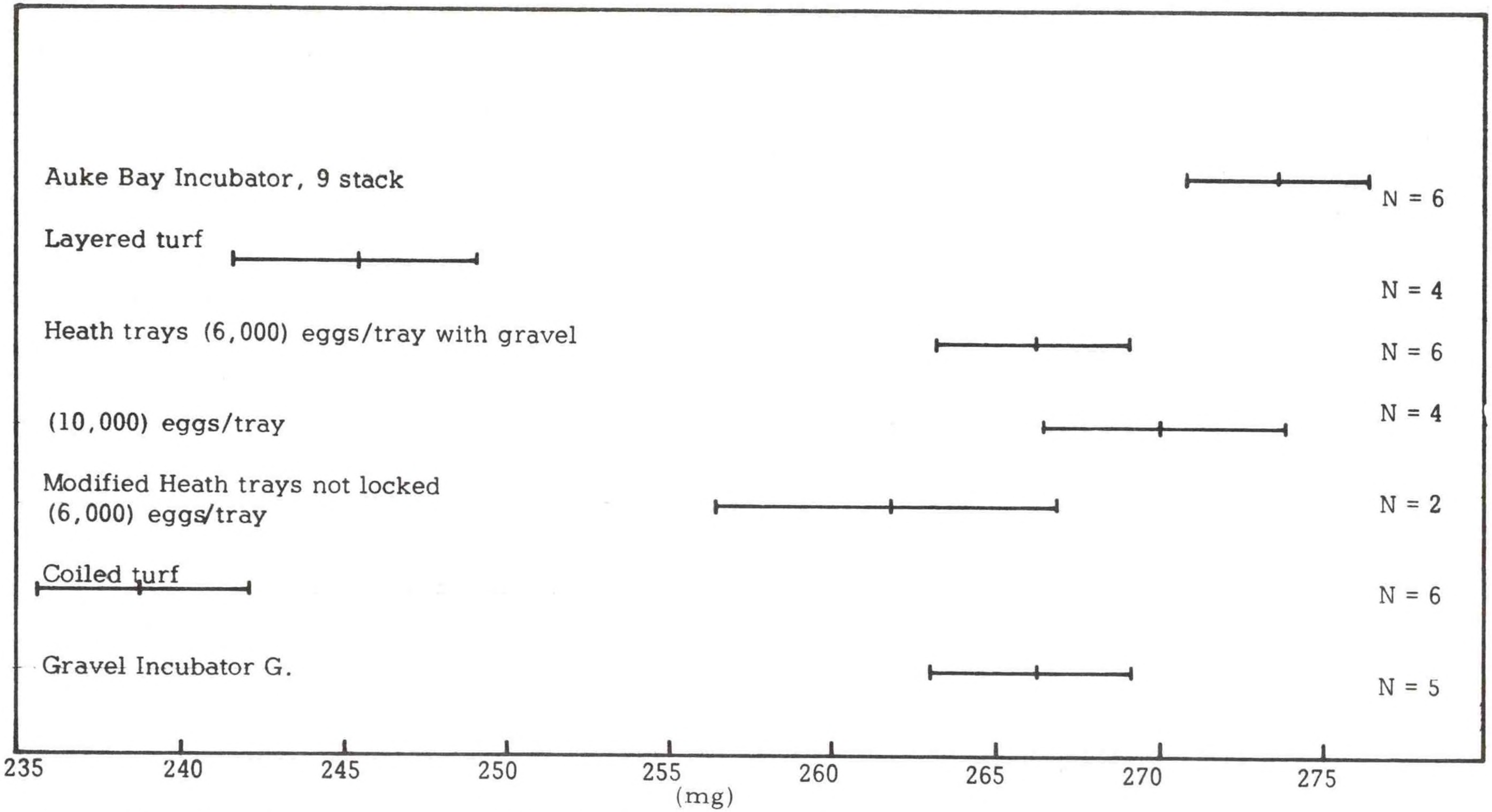

Figure 6.--Wet weights of unfed pink salmon fry after incubating eggs from the same parents in incubators of varying designs; the length of each line represents ± two times the standard deviation of pooled means for N samples of 50 fry per sample.

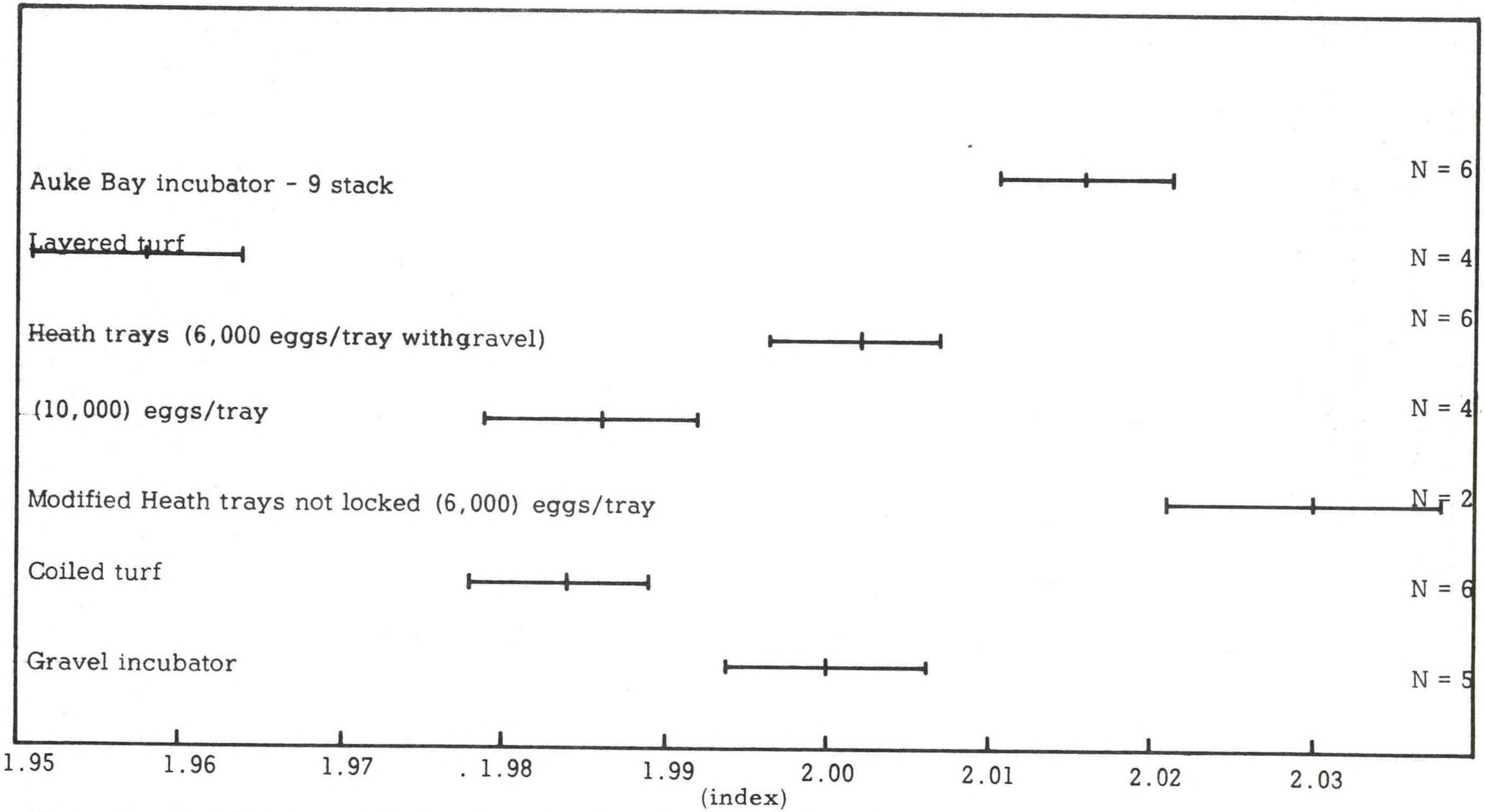

Figure 7.--Developmental indices, K_D, of unfed pink salmon fry after incubating eggs from the same parents in incubators of varying designs; the length of each line represents ± two times the standard deviation of pooled means for N samples of 50 fry per sample.

causing a large number of alevins to die. Fungus masses formed around these dead alevins channelizing the flow of water and triggering additional mortalities. Alevins in the layered turf incubator gravitated to the bottom two layers of turf immediately after hatching. The alevins remained crowded at the bottom of the incubator until the last two weeks of their incubation period. Improper control of light was believed to be the factor responsible for keeping these alevins on the bottom of the incubator.

Seeding Density and Flow

In order to evaluate the effects of seeding density and flow rate on the physical features--length and weight--of alevins produced in the incubators, response surface theory of applied statistics was utilized. If the physical measurement is called Y, we consider Y as a function of two variables, seeding density, X_1, and flow rate, X_2. A graph of the response Y above the (X_1, X_2)-plane can be thought of as a surface. Presumably this surface will be smooth and well behaved; if so, a quadratic surface may adequately approximate the actual surface.

Algebraically, the quadratic surface is:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + \varepsilon$$

where

$\beta_o, \beta_1, \beta_2, \beta_{11}, \beta_{22}$, and β_{12} are constants,

X_1 is the seeding density,

X_2 is the flow rate,

Y is the physical measurement,

and ε is a residual due to lack of fit of the quadratic surface, to random variation in growth of fry in incubators treated identically, and variation in growth of fry among subsamples from an incubator.

An experiment was conducted by selecting ten (X_1, X_2) pairs, incubating pink salmon eggs in Model 2 Auke Bay incubators at these conditions, and measuring the response, either length or weight, from a sample of emerging alevins. Points in the (X_1, X_2) plane were selected in a hexagonal rotatable design with four center points (Box and Hunter, 1958; also see Table 5). This design has the desirable feature of gathering a constant quantity of information in any direction from the center of the design though the quantity varies with the distance from the center (Box and Hunter, 1958). The four replicates at the center of the design (40 eggs/in^2, 5.5 gpm) provide a measure of experimental error with which to test the adequacy of the quadratic model in approximating the actual surface.

When length is considered as the response, the test for lack of fit was not significant even at the 10% testing level (Table 6). Therefore, no strong evidence is provided by the experiment to suggest the approximation of the actual surface by the quadratic surface was inadequate. The test for second order terms, β_{11}, β_{22}, and β_{12} was not significant even at the 25% level Table 6). Therefore, curvilinearity is not detectable in the surface over the (X_1, X_2) region included in the experiment. Even the first order terms are not significant at the 5% level of testing although the test attains significance at the 10% level (Table 6).

If the first order model is adopted,

$$Y = 32.80 - .014\, X_1 + .009\, X_2,$$

the experiment indicates length decreases with seeding density and increases with flow rate. Over the range of densities and flows covered by our study, we expect length to decrease .014 mm. per increase of an egg/in.^{2} in density if flow rate is held constant. Length is predicted to increase by .009 mm. per increase of 1 gpm in flow if density is fixed.

Suppose a given pair $(\underset{\sim}{X}_1, X_2)$ gives a desired length, but we wish to change the seeding density to X_2. To maintain the alevin length, flow would have to be altered to $X_2 + 1.56\,\Delta X_1$ where ΔX_1 is the change in seeding density and X_2 is the initial flow.

Densities of alevins changed during the experiment as fry emigrated from several incubators before completion of sampling for size. If the regression analysis above is performed using densities of fry at completion of the study, neither the test for lack of fit, first order, or second order terms is significant at even the 10% level of testing. The overall mean of the observations as a model to describe length is not significantly improved upon by adding density and flow as explanatory variables. For comparison with the preceding analysis, the first order model using densities at completion of the experiment is

$$Y = 32.461 - .00575\, X_1 + .004\, X_2.$$

Considering growth in weight as the response, the analysis using initial seeding densities and flow rates as explanatory variables indicated again the quadratic surface was an adequate approximation to the actual surface (Table 7). In contradistinction to the length analysis, however, both first and second order terms were significant in the regression of weight on explanatory variables (Table 7). The complete model describing weight of alevins produced as related to seeding density and flow rate is

$$Y = 286.0 - 0.352\,X_1 - 1.125\,X_2 + 0.001\,X_1^2 + 0.062\,X_2^2 + .019\,X_1\,X_2.$$

Loci of points in the (X_1, X_2) plane at which weight of alevins is predicted by the model to equal 278, 275, 273, 271, 269, and 268 mg have been determined (Figure 8). Values for weight at (X_1, X_2) pairs not lying on the isopleths can be obtained by interpolation. Weight of alevins diminishes with increasing seeding density at a given flow rate. At low seeding levels, flow has little effect on size; however, at the higher seeding levels of the experiment, increased flow resulted in larger alevins.

Table 5.--Hexagonal rotatable design in seeding level, X_1, and flow rate, X_2, to evaluate response, Y- length or weight--of alevins produced at the two factor combinations.

Incubator number	Factor Levels seeding level (X_1) (seeds/in.^{2})	Flow (X_2) (gpm)	Response (Y) Length (mm.)	Response (Y) Weight (mg.)
8	6	5.5	32.28	279.0
9	57	8.0	32.23	271.5
6	57	3.0	32.08	268.4
7	40	5.5	32.24	272.7
3	40	5.5	32.42	273.5
4	23	8.0	32.39	278.0
2	23	3.0	32.51	278.1
10	74	5.5	32.09	270.0
5	40	5.5	32.32	272.0
1	40	5.5	32.26	275.1

Table 6.--Regression analysis of length as related to seeding level and flow.

Source	df	SS	MS	F
First order terms	2	.07864	.03932	6.0 ns
Second order terms	3	.04161	.01387	2.1 ns
Lack of fit	1	.02692	.02692	4.1 ns
Experimental Error	3	.01960	.00653	
Total	9	.16676		

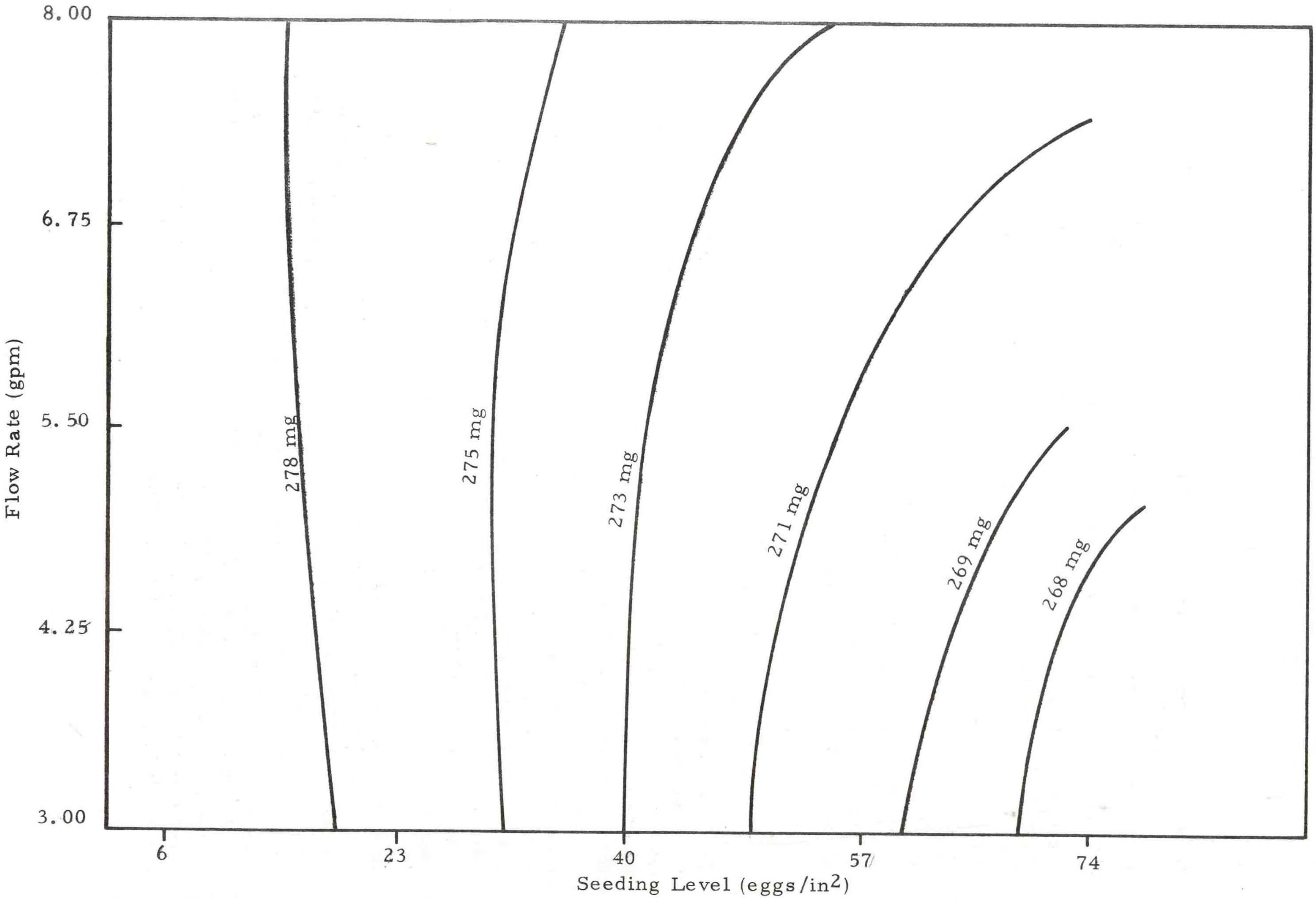

Figure 8.--Average weight of alevins as related to seeding level and flow rate determined by surface response analysis.

Table 7.--Regression analysis of weight as related to seeding level and flow

Source	df	SS	MS	F
First order terms	2	99.7407	49.87035	28.08*
Second order terms	3	168.8470	56.28233	31.69**
Lack of fit	1	8.6548	8.65480	4.87ns
Experimental error	3	5.3275	1.77583	
Total	9	282.5707		

* significant at 5% level of testing

** significant at 1% level of testing

ns not significant at 5% level of testing

Time of Emergence

The timing of release of unfed hatchery fry, to begin the marine phase of their life, should coincide as closely as possible with the natural timing of seaward migration of wild fry. Fry that complete their development to the "buttoned-up" or migratory stage too rapidly may migrate to sea before the occurrence of spring warming or zooplankton blooms. Then, because of slow growth, they could be exposed to their usual high rate of predation for an unnaturally long period of time. The result would be low ocean survival and a scarcity of returning adults. Two solutions are suggested, i.e., feed the fry in the hatchery until estuarine conditions and fry size favor their survival or design incubation systems that will produce properly timed emergence and migration of unfed fry. We advocate the latter solution because we believe it would be the most economical.

At Auke Creek we compared the timing of release of fry from various types of incubators with timing of seaward migration of wild fry as indexed by a fyke net. Practically all of the hatchery fry migrated seaward in April 1975, whereas less than one-half of the creek fry migrated seaward by the end of April and the remainder migrated seaward in May. Less than 4% of either hatchery or creek fry migrated prior to April 1. Fry attained the buttoned up fry stage of development earlier in artificial turf incubators than in gravel incubators. Of the two basic types of turf incubators, horizontal flow and upwelling flow, emergence or release of fry was closer to an acceptable pattern for the upwelling flow incubators.

Fry produced in gravel incubators emerged to migrate seaward about 12 days earlier than fry produced by natural spawning in Auke Creek. Emergence from gravel incubators lasted from February 28 until May 23, while the wild fry outmigration began February 26 and ended May 29, 1975 (Figure 9).

In previous operations at the Auke Creek hatchery, gravel incubator fry have emerged 2 to 6 weeks ahead of creek fry. The early emergence of incubator fry was attributed to the net effect of differences in water temperature in the hatchery and the creek. This year, however, the hatchery temperature averaged 1.5^{o}C. lower than usual and the net difference between hatchery and creek temperatures does not completely explain the early emergence of gravel incubator fry. Instead, the egg collection schedule appears to explain the observed difference in timing. Each of the gravel incubators was seeded with eggs as soon as 150,000 eggs reached the eyed stage of development. After seeding four of the gravel incubators, the remaining eyed eggs were seeded into a fifth gravel incubator, Incubator G; the experimental turf incubators; and Heath incubator trays with gravel. As a result of this seeding schedule, the gravel incubator fry originated from the first 60% of the eggs spawned whereas the creek fry are presumed to have originated from all segments of the natural spawning. A graphical analysis of timing of emergence shows the wild fry emerging somewhat later, about 2 weeks later, than gravel incubator fry. The mean incubation period; that is, number of days from fertilization to emergence, was almost the same for these two groups of fry. The incubation period was 231 days for gravel fry (range 227-238 days) and 232 days for creek fry (Table 8).

Pink salmon eggs in the coiled turf and layered turf incubators had incubation periods of 220 and 225 days, respectively. Fry reared in the Heath trays and in the Auke Bay incubators were not allowed to emerge voluntarily. Fry in the Heath trays were examined for visible yolk and closure of the ventral slit and all fry were released on April 4, when it was decided that they were fully developed. The lock-in screens in the Auke Bay incubators were removed on April 15, and the fry immediately began to leave the incubators (Figure 10). "Buttoned-up" fry were produced after 201 days incubation in the Heath trays and 214 days in the Auke Bay incubators. The Auke Bay incubator fry migrated seaward 2 weeks ahead of fry from a gravel incubator, Incubator G, containing eggs from the same parents. Heath tray fry, also from the same parents, migrated seaward 4 weeks ahead of fry from gravel incubator G.

Horizontal-flow incubators using either gravel or turf were less satisfactory than upwelling flow incubators using gravel for the properly timed production

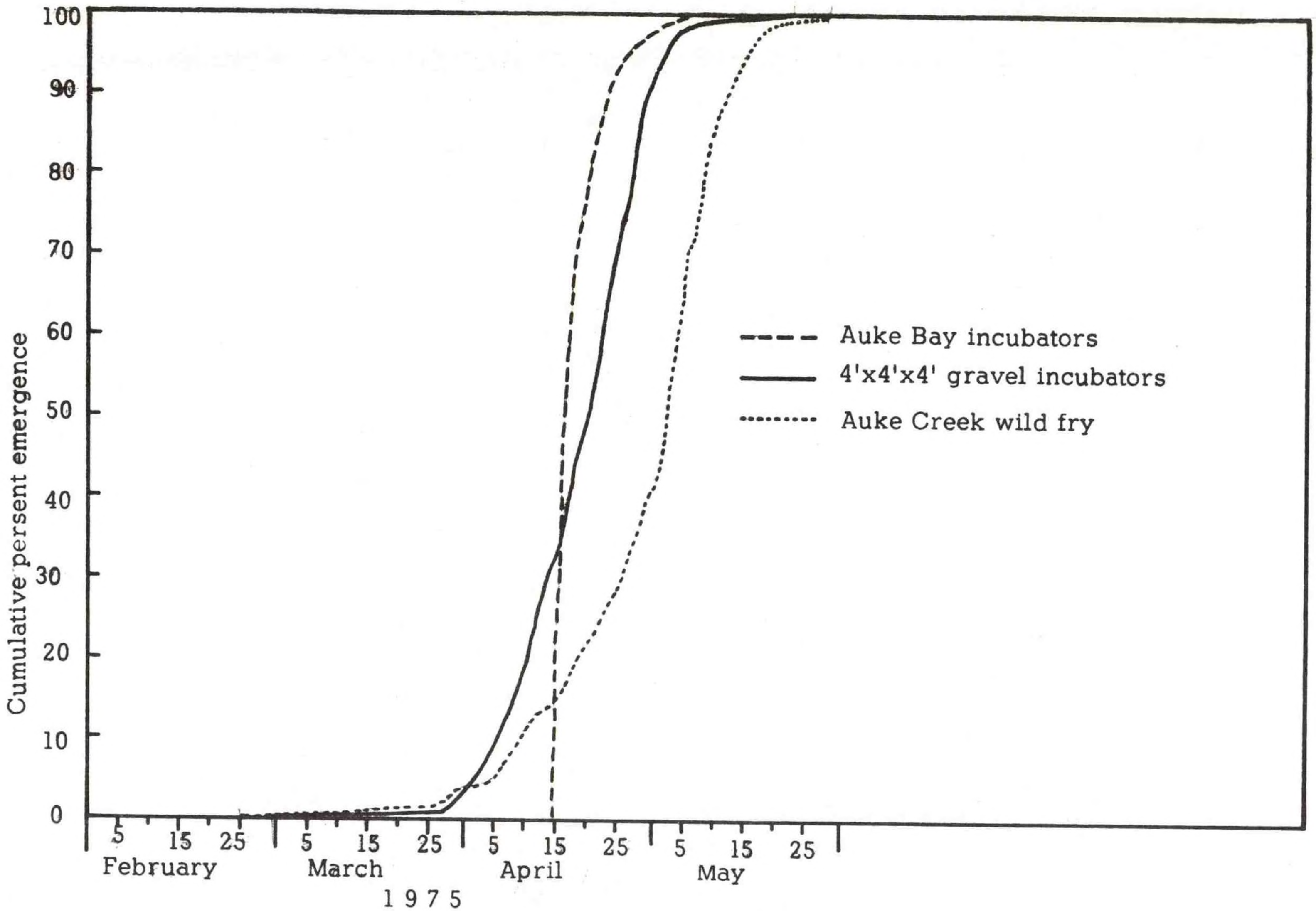

Figure 9.--Daily cumulative percent emergence of pink salmon fry from 19 of the Auke Bay Incubators, 5 of the 4'x4'x4' gravel incubators, and Auke Creek.

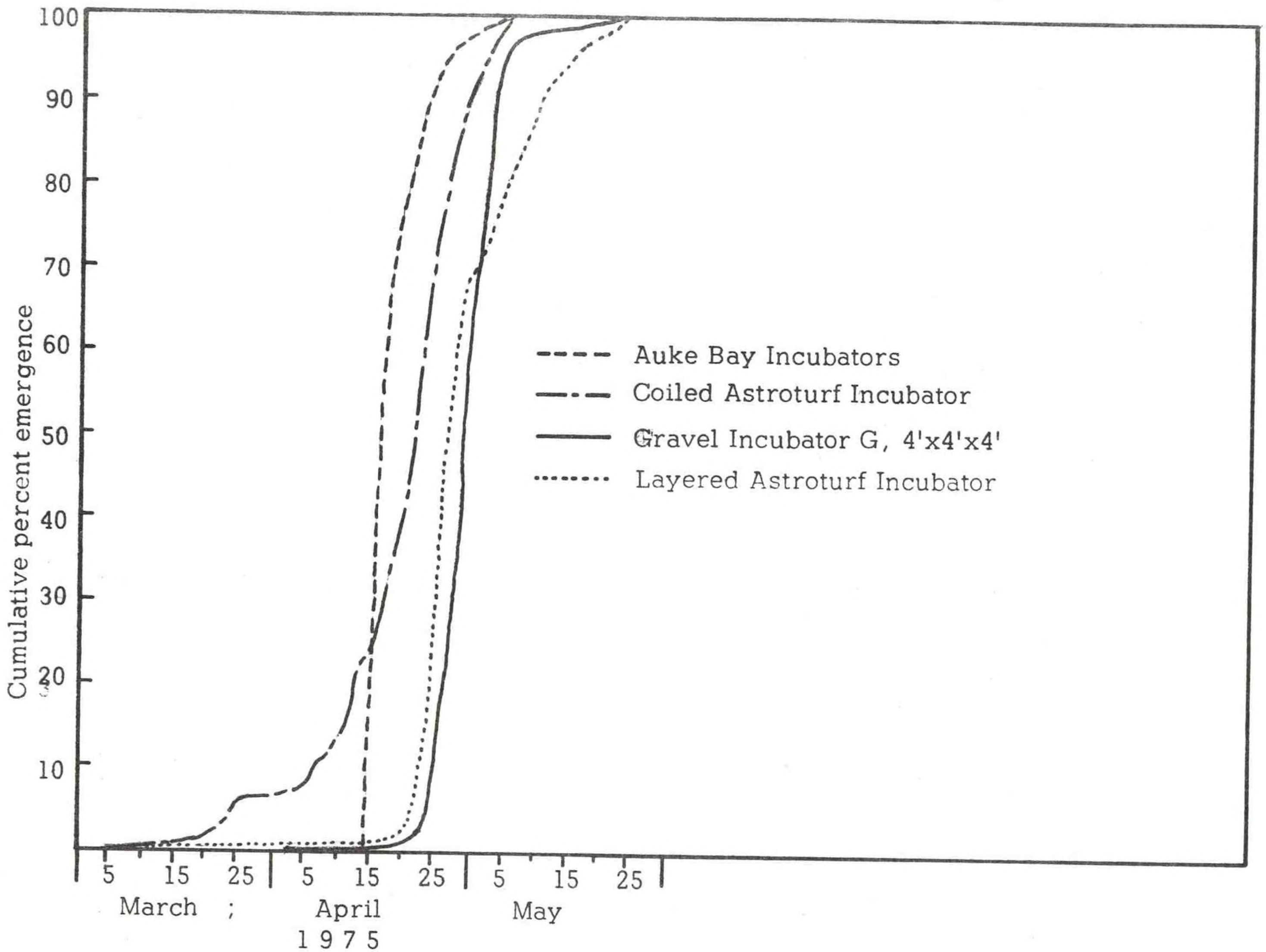

Figure 10.--Daily cumulative percent emergence of pink salmon fry from a coiled Astroturf incubator, 4'x4'x4' gravel Incubator G, a layered Astroturf incubator, and Auke Bay Incubators.

of large, properly developed, unfed fry. Horizontal-flow incubators, as defined in this report, obligate hatchery managers to hold and feed their fry for several weeks before release into estuaries. This is basically the Japanese and the Netarts systems of salmon enhancement. Fry quality; that is, ocean survival, of these fry has never been directly compared with wild fry.

There may be an alternative to obligate feeding of fry from turf incubators. Plastic turf has not been adequately tested in upwelling-flow incubators. Various combinations of turf depth, turf orientation, egg density, and water velocity will be tested with the 1975 brood pink salmon at Auke Creek in an effort to produce properly timed, unfed fry of acceptable size and survival ability.

Table 8.--Incubation periods for 1974 brood pink salmon eggs in various environments at Auke Creek

Incubator	Date of 50% fertilization	Date of 50% emergence	Number of days	Volitional emergence
Gravel Boxes:				
A	Aug. 21	April 16	238	yes
B	Aug. 27	April 12	228	yes
C	Sept. 1	April 21	232	yes
D	Sept. 9	April 25	228	yes
G	Sept. 15	April 30	227	yes
Heath trays w/gravel not locked in	Sept. 15	April 4	201	no
Heath trays w/gravel locked in	Sept. 15	April 4	201	no
Auke Bay incubator Stack of 9	Sept. 15	April 17	214	no
Auke Bay incubator density flow expt.	Sept. 15	April 17	214	no
Coiled turf incubator	Sept. 15	April 23	220	yes
Layered turf incubator	Sept. 15	April 28	225	yes
Wild fry	Sept. 13	May 3	232	yes

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